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(21) International Application Number: PCT/EP89/01180 (22) International Filing Date: 3 October 1989 (03.10.89) (30) Priority data: 252,765 3 October 1988 (03.10.88) US (71) Applicant: STICHTING REGA VZW [BE/BE]; Minderbroedersstraat 10, B-3000 Leuven (BE). (72) Inventors: BALZARINI, Jan, Maria, René ; Prosper Poulletlaan 10/9, B-3000 Leuven (BE). DE CLERCQ, Erik, Désiré, Alice ; Paul Lebrunstraat 35/7, B-3000 Leuven (BE). VAN AERSCHOT, Arthur, Albert, Edgard ; Heist Goorstraat 29, B-3100 Heist o/d Berg (BE). HERDEWIJN, Piet, André, Maurits, Maria ; Diestsesteenweg 232, B-3200 Kessel-lo (BE).	(74) Agents: BRUIN, Cornelis, Willem et al.: Octrooibureau Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>	
(54) Title: 5-HALOGENO-3'-FLUORO-2',3'-DIDEOXYURIDINE COMPOUNDS AND THEIR THERAPEUTIC APPLICATION (57) Abstract The novel 5-halogeno-3'-fluoro-2',3'-dideoxyuridines particularly FddCIUrd, FddBrUrd, FddIUrd and FddFUrd proved antivirally effective in several <i>in vitro</i> systems (i.e. HIV-1 replication in MT4 and HUT-78 cells and HIV-2 replication in MT4 cells). Of these FddUrd derivatives FddCIUrd is endowed with the highest selectivity index <i>in vitro</i> , comparable to that AzddThd in MT4 cells. These observations make the members of the 5-halogeno-substituted FddUrd derivatives attractive antivirals agents for the treatment of AIDS, AIDS-related diseases and retroviral diseases including hepatitis B.		

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5-Halogeno-3'-fluoro-2',3'-dideoxyuridine compounds and their therapeutic application

This invention relates to novel 5-halogeno-3'-fluoro-2',3'-dideoxyuridine compounds, namely 5-chloro-3'-fluoro-2',3'-dideoxyuridine (FddClUrd), 5-bromo-3'-fluoro-2',3'-dideoxyuridine (FddBrUrd), 5-iodo-3'-fluoro-2',3'-dideoxyuridine (FddIUrd), and 5-fluoro-3'-fluoro-2',3'-dideoxyuridine (FddFUrd) and to their application as a novel therapeutical agent for treating AIDS, AIDS-related diseases and other retroviral diseases including hepatitis B.

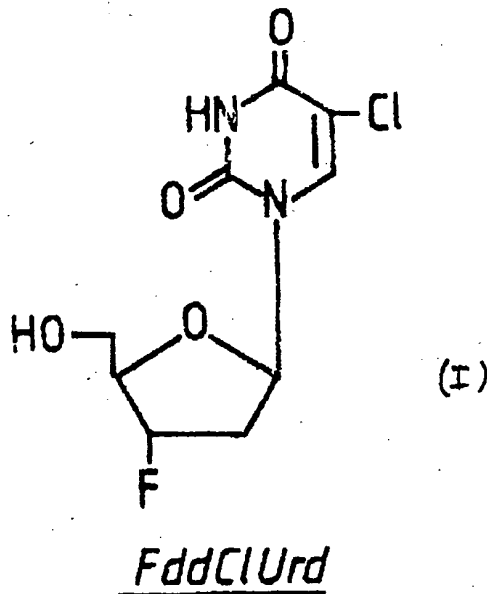
Since the advent of the acquired immunodeficiency syndrome (AIDS), many efforts are conducted world-wide to develop novel and selective inhibitors of human immunodeficiency virus (HIV) replication in vitro. 2',3'-Dideoxynucleoside analogues belong to a attractive class of anti-HIV compounds, several members of which are currently subject to clinical trials (i.e. 3'-azido-2',3'-dideoxythymidine (AzddThd, AZT, Retrovir^R), 2',3'-dideoxycytidine (ddCyd), 2',3'-dideoxyadenosine (ddAdo), 2',3'-dideoxyinosine (ddIno)] (for an overview, De Clercq, E. Perspectives for the chemotherapy of AIDS. Anticancer Res. 7: 1023-1038 (1987); De Clercq E. et al., Anti-HIV-1 activity of 2',3'-dideoxynucleoside analogues: structure-activity relationship. Nucleosides & Nucleotides, in press (1988). Recently, we synthesized the 3'-fluoro-substituted derivatives of 2',3'-dideoxyuridine (FddUrd), 2',3'-dideoxythymidine (FddThd), 5-ethyl-2',3'-dideoxyuridine (FddEtUrd) and ddCyd (FddCyd) and compared their antiretroviral activities and antimetabolic properties (Balzarini, J. et al., Anti-retrovirus

activity of 3'-fluoro- and 3'-azido-substituted pyrimidine
2',3'-dideoxynucleoside analogues. Biochem. Pharmacol.

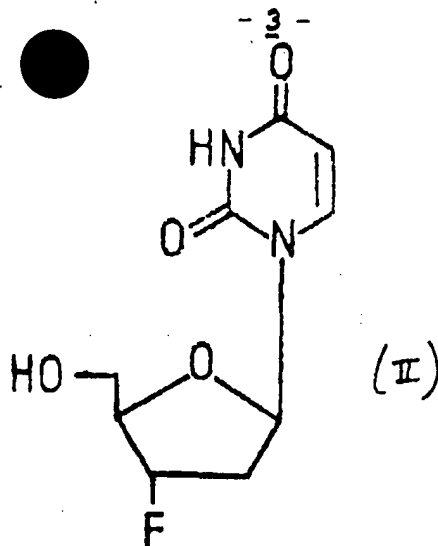
37: 2847-2856 (1988). We found that FddThd and FddUrd belonged
to the most potent anti-HIV compounds among the pyrimidine
5 2',3'-dideoxynucleoside analogues.

During extensive research for new potential
inhibitors of AIDS, AIDS-related diseases and retroviral
diseases we found that 5-halogeno-substituted FddUrd
derivatives were potent inhibitors of HIV-1 and HIV-2
10 replication in MT4 and HUT-78 cells [50% effective dose
(ED₅₀) for HIV-1 in MT4 cells: 0,1-0,5 μ M]. Moreover,
due to its poor cytostatic activity, FddClUrd [50%
cytostatic dose (CD₅₀): 535 μ M] emerged as the most
selective anti-HIV agent among the 5-halogeno-substituted
15 FddUrd derivatives.

5-Chloro-3'-fluoro-2',3'-dideoxyuridine
(FddClUrd) represented by the following chemical
formula (I)



was synthesized by acylating 3'-fluoro-2',3'-dideoxy-
uridine (FddUrd) represented by the following chemical
35 formula (II)



FddUrd

with acetic anhydride in pyridine for 2 h at room temperature, after which the mixture was evaporated and coevaporated twice with toluene to remove excess anhydride and acetic acid. The residue was taken up in pyridine, 2 eq of chlorosuccinimide were added and the mixture heated for 45 min at 100°C (colouring dark brown). Evaporation yielded an oil which was treated overnight at room temperature with a saturated solution of ammonia in methanol. Evaporation resulted in a brown foam which was purified by column chromatography yielding FddClUrd. (Reaction with chlorosuccinimide in glacial acetic acid affords the title compound in lower yield). Physical properties of FddClUrd are as follows:

¹³C NMR (DMSO-d₆) δ: 37.7 (C-2', 20.8 Hz); 60.8 (C-5', 9.8 Hz); 84.8 (C-1'); 85.3 (C-4', 23.2 Hz); 94.7 (C-3', 174.6 Hz); 107.6 (C5); 137.5 (C-6); 149.5 (C-2); 158.9 (C-4).

¹H NMR (DMSO-d₆) δ: 2.01-2.63 (m, H-2' + H-2''); 3.65 (m, H-5' + H-5''); 4.21 (dt, H-4', J = 3.5 Hz, J_{4',F} = 27.3 Hz); 5.30 (t, 5'-OH, exchangeable D₂O); 5.31 (dd, H-3', J = 4 Hz, J_{3',F} = 54.4 Hz); 6.18 (t, H-1', J = 7.2 Hz); 8.24 (s, H-6); 11.80 (brs, N-H, exchangeable D₂O).

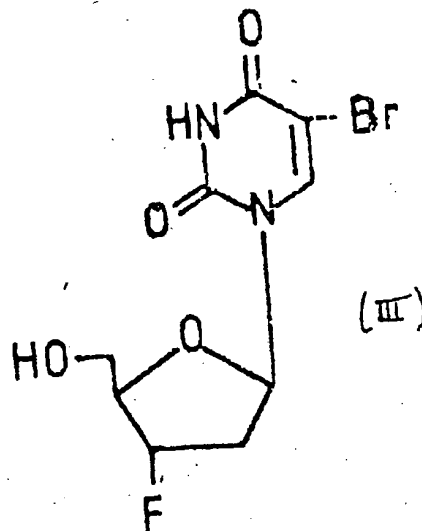
mp. (acetone-PE) : 181°C (melt. + dec.)

C₉H₁₀O₄N₂FCl 264.65

MS : 264 (M⁺), 147 (B+H₂), 146 (B+H), 119 (sugar, 100%), 99 (sugar-HF).

UV : 276.5 nm (ε = 8650).

5-Bromo-3'-fluoro-2',3'-dideoxyuridine
(FddBrUrd) represented by the following chemical
formula (III)



FddBrUrd

was prepared according to two different procedures. In the first procedure, the same modus operandi was followed as for the synthesis of FddClUrd, except that 2 eq of bromosuccinimide were added and the reaction performed in either glacial acetic acid or preferably in anhydrous pyridine. In the second procedure, FddUrd was dissolved in pyridine and 1.3 eq of a bromine solution in carbon tetrachloride were added. The reaction was stirred for 2 h at room temperature when TLC indicated complete reaction. Evaporation and chromatographic purification afforded the title compound in excellent yield. Physical properties of FddBrUrd are as follows:

^{13}C NMR (DMSO- d_6) δ : 37.9 (C-2', 19.5 Hz); 51.3 (C-5); 60.8 (C-5', 12.2 Hz); 84.8 (C-1'), 85.3 (C-4', 23.2 Hz); 94.8 (C-3', 174.6 Hz); 140.1 (C6); 149.9 (C-2); 159.1 (C-4).

^1H NMR (DMSO- d_6) δ : 2.27-2.60 (m, H-2' + H-2''); 3.64 (m, H-5' + H-5''); 4.20 (dt, H-4', $J_{4',F} = 26.3$ Hz); 5.30 (t, 5'-OH); 5.31 (dm, H-3', $J_{3',F} = 53.6$ Hz); 6.17 (dt, H-1'); 8.32 (s, H-6); 11.81 (brs, N-H).

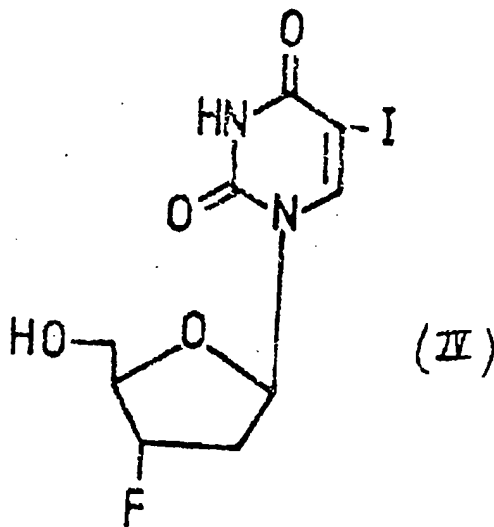
mp. (MeOH-EtOAc) : 154-155°C (dec.)

$\text{C}_9\text{H}_{10}\text{N}_2\text{O}_4\text{FBr}$ 309.1

MS : 308 (M^+), 191 ($\text{B}+\text{H}_2$), 190 ($\text{B}+\text{H}$), 119 (sugar, 100%), 99 (sugar-HF).

UV : 278 nm ($\epsilon = 8650$).

20 5-Iodo-3'-fluoro-2',3'-dideoxyuridine
(FddIUrd) represented by the following chemical formula
(IV)



FddIUrd

was prepared from FddUrd. Therefore,

FddUrd was dissolved in methanol and 1.5 eq of a stock solution of iodine monochloride in methanol were added. The mixture was heated at reflux temperature for 3 h and subsequently evaporated, and coevaporated several times with methanol. Chromatographic purification afforded the title compound in moderate yield. Physical properties of FddIUrd are as follows:

^{13}C NMR (DMSO- d_6) δ : 37.8 (C-2', 20.8 Hz); 60.8 (C-5', 9.8 Hz); 69.9 (C-5); 84.6 (C-1'); 85.2 (C-4', 23.2 Hz); 94.8 (C-3', 173.3 Hz); 144.8 (C-6); 150.1 (C-2); 160.4 (C-4).

^1H NMR (DMSO- d_6) δ : 2.06-2.63 (m, H-2' + H-2''); 3.65 (m, H-5' + H-5''); 4.19 (dt, H-4', $J_{4'}$, F = 27.3 Hz); 5.27 (t, 5'-OH); 5.29 (dm, H-3', $J_{3'}$, F = 53.9 Hz); 6.16 (dt, H-1'); 8.34 (s, H-6); 11.69 (brs, N-H).

mp. (MeOH-EtOAc) : 159-160°C (dec.)

$\text{C}_9\text{H}_{10}\text{N}_2\text{O}_4\text{FI}$ 356.1

MS : 356 (M^+), B+H (238, 100%), 119 (sugar), 99 (sugar-HF).

UV : 284 nm (broad max) (Σ = 7200).

FddFUrd was prepared using similar preparative methods.

FddClUrd, FddBrUrd, and FddIUrd will be compared with FddUrd, FddThd and AzddThd. The latter known compounds were prepared according to previously published methods (Herdewijn, P. et al. 3'-Substituted 2',3'-dideoxy-nucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. J. Med. Chem. 30; 1270-1278 (1987); Horwitz, J.P. et al. Nucleosides. V. The monomesylates of 1-(2'-deoxy- β -D-lyxofuranosyl)thymine. J. Org. Chem. 29; 2076-2078 (1964); Herdewijn, P. et al. Synthesis of nucleosides fluorinated in the sugar moiety. The application of diethylaminosulfur trifluoride to the synthesis of fluorinated nucleosides. Nucleosides & Nucleotides, in press (1988).

All reagents used were of the highest quality obtainable.

Radiochemicals. (Methyl-³H)dThd (specific radioactivity 40 Ci/mmol) and (5-³H)dCyd (specific radioactivity 20 Ci/mmol) were obtained from the Radiochemical Centre Amersham (Amersham, U.K.), whereas
5 (5-³H)dUrd was from ICN Pharmaceuticals (Irvine, CA).

Cells. MT4, HUT-78, Raji/O and Raji/TK⁻ cells (a dThd kinase-deficient mutant cell line derived from wild-type Raji/O cells) were grown as described previously
10 (Balzarini, 1988). Characterization of the Raji/O and Raji/TK⁻ cells has been described earlier (Balzarini, J. et al. Role of thymidine kinase in the inhibitory activity of 5-substituted-2'-deoxyuridines on the growth of human and murine tumor cell lines. Biochem. Pharmacol.
15 31:1089-1095 (1982)). Molt/8 cells were cultured in the same medium as described for MT4.

Viruses. HTLV-III_B (designated HIV) were derived from a pool of American patients with AIDS, and
20 obtained from the supernatant of HIV-infected H9 cell cultures (Popovic, M. et al. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497-500 (1984). LAV-2 (designated HIV-2) was obtained from
25 Dr. L. Montagnier, Paris, France. Moloney murine sarcoma virus (MSV) was prepared from tumors induced by in vivo infection of 3 days old NMRI mice according to the procedure described by De Clercq, E. et al. Moloney sarcoma virus-induced tumors in mice: inhibition or stimulation by
30 (PolyrI).(polyrC). Proc. Soc. Exp. Biol. Med. 137:590-594 (1971).

Therapeutic compositions containing FddClUrd, FddBrUrd, or FddIUrd respectively as an active ingredient for treating AIDS in human practice may take the form of
35 powders, suspensions, solutions, sprays, emulsions, unguents or creams and may be used for local application, for intranasal, rectal, vaginal and also for oral or parenteral

(intravenous, intradermal, intramuscular, intrathecal etc.) administration. Such compositions may be prepared by combining (e.g. mixing, dissolving etc.) the active ingredient with pharmaceutically acceptable excipients of neutral character (such as aqueous or non-aqueous solvents, stabilizers, emulsifiers, detergents, additives), and further, if necessary with dyes and aromatizers. The concentration of the active ingredient in the therapeutic composition may vary widely between 0.1% and 100%, dependent on the mode of administration. Further, the dose of the active ingredient to be administered may vary between 0.1 mg and 100 mg per kg of body weight.

The pharmaceutical properties of the novel 5-halogeno derivatives of FddUrd, notably their anti-retroviral effects, especially HIV, are documented by the following examples which should not be read in a restrictive sense.

Example 1

Inhibitory effects of FddClUrd, FddBrUrd and FddIUrd on HIV-1-induced cytopathogenicity in MT4 cells.

Human T-lymphocyte MT4 cells (5×10^5 cells/ml) were suspended in fresh RPMI-1640 culture medium (Gibco) containing 10% v/v fetal calf serum (Gibco), 2 mM L-glutamine (Flow Laboratories), 20 mM Hepes buffer, 0.075% (w/v) NaHCO_3 (Flow Laboratories), 2.5 $\mu\text{g/ml}$ Fungizone (Squibb N.V., Brussels, Belgium) and 20 $\mu\text{g/ml}$ Geomycine (Essex N.V., Heist-o/d-Berg, Belgium), and infected with 200 CCID₅₀ (cell culture infective dose-50) HIV-1 or HIV-2 per ml cell suspension. Then 100 μl of the infected cell suspension is added to 100 μl of an appropriate dilution of test compound in 200 μl microplate wells of a Flat Bottom Microtest III Plate (Falcon, Becton Dickinson, Oxnard, CA) (i.e. 20 CCID₅₀ HIV/200 μl well/ 5×10^4 cells), and further incubated at 37° in a CO₂-controlled humidified atmosphere. After incubation for 5 days, viable cell counts were determined for both virus-infected cell cultures and non-infected cell cultures (which had been incubated with the

same concentration of compounds as the virus-infected cells). The 50 % effective dose (ED₅₀) and 50 % cytotoxic dose (CD₅₀) were defined as the compound concentration required to reduce by 50 % the number of viable cells in the virus-infected and non-infected cell cultures, respectively.

As shown in Table 1 and the single Figure, as a rule, FddClUrd, FddBrUrd and FddIUrd were 4- to 10-fold less active as antiviral agents than the parent compound FddUrd. Their 50 %-effective doses (ED₅₀) ranged between 0.16 and 0.41 μ M. The 5-halogeno-substituted FddUrd derivatives were also effective against HIV-2 replication in MT4 cells (data not shown). However, marked differences were noted in the cytotoxic properties of the compounds against MT4 cells. With a CD₅₀ (50 %-cytotoxic dose) of 1.0 and 2.2 μ M, FddUrd, and FddIUrd were the most potent, and with a CD₅₀ of 535 μ M, FddClUrd was the least potent inhibitor of MT4 cell proliferation. FddBrUrd proved modestly cytotoxic to MT4 cells (CD₅₀ : 24 μ M). Consequently, the selectivity index (S.I.) of the 5-halogeno-substituted FddUrd derivatives varied markedly from one to the other. FddClUrd was endowed with the greatest S.I. (1446) and FddIUrd had the lowest S.I. (14). Thus, FddClUrd is remarkably superior to the other 5-halogeno-substituted FddUrd derivatives in its anti-HIV-1 activity. Although 100-fold more effective than FddClUrd in inhibiting the cytopathogenicity of HIV-1 in MT4 cells, AzddThd proved also \sim 100 fold more cytotoxic for the uninfected MT4 cells than FddClUrd. Consequently, FddClUrd had a S.I. comparable to that of AzddThd. On the other hand, FddThd, which is an extremely effective agent against HIV replication in MT4 cells, was at least 1000-fold more toxic than FddClUrd, resulting in a 7-fold lower selectivity index than that observed for FddClUrd.

Table 1. Antiretroviral effects of 5-halogeno-substituted FddUrd analogues

Compound	HIV-induced cytopathogenicity in MT-4 cells		HIV-induced expression of viral antigens in HUT-78 cells		MSV-induced transformation of C3 cells		
	ED ₅₀ ^a (μM)	CD ₅₀ ^b (μM)	S.I. ^c	ED ₅₀ ^a (μM)	ED ₅₀ ^a (μM)	MCC ^d (μM)	S.I.
FddClUrd	0.38 ± 0.06	535 ± 41	1446	1.6	457 ± 7	>500	>1.1
FddBrUrd	0.41 ± 0.16	24 ± 18	59	2.4	313 ± 13	>500	>1.4
FddIUrd	0.16 ± 0.1	2.2 ± 2.0	14	7.9	>100	±500	<5
FddUrd	0.06 ± 0.02	1.1 ± 0.2	25	0.8	>500	>500	-
FddThd	0.001 ^e	0.197 ^e	197 ^e	-	0.06 ^e	>500	>8333
AzddThd	0.003 ± 0.001	4.81 ± 2.53	1603	0.038	0.02 ^e	>500	>25000

^a 50% antiviral effective dose, required to affect a 50% reduction in the cytopathic effect of HIV-1 in MT-4 cells, HIV-1 antigen expression in HUT-78 cells, or transformation of C3 cells by MSV.

^b 50 % cytotoxic dose required to reduce the number of viable cells in the untreated MT-4 and HUT-78 cell cultures.

^c Selectivity index or ratio of CD₅₀/ED₅₀ or MCC/ED₅₀.

^d Minimum cytotoxic concentration. The parameter followed here was an alteration of normal cell morphology.

^e Data taken from Balzarini et al (1988)

Example 2

Effect of dThd, dCyd and Urd on the anti-HIV activity of the 5-halogeno-substituted FddUrd derivatives in MT4 cells.

MT4 cells (10^6 cells/ml) were suspended in culture medium as described above, and infected with 200 CCID₅₀ HIV-1 per ml cell suspension. The 50 μ l of the infected cell suspension was added to 100 μ l of an appropriate dilution of test compound and 50 μ l of medium containing 25 μ M dThd, 1000 μ M dCyd or 250 μ M dThd + 1000 μ M dCyd in 200 μ l microplate wells. After incubation of 5 days, viable cell counts were determined as described above. If intracellular phosphorylation by dThd kinase of FddUrd and its 5-halogeno-substituted congeners is a prerequisite for their anti-retrovirus and cytotoxic activity, the addition of high concentrations of dThd should prevent the biological activity of the FddUrd derivatives. Indeed, addition of 250 μ M dThd (in the presence of 1000 μ M dCyd to avoid cytotoxicity of dThd) resulted in a marked decrease of the anti-HIV-1 activity of FddUrd and its 5-halogeno-substituted congeners by more than 2 to 3 orders of magnitude (Table 2). Also, a dramatic decrease of the selectivity index was observed. Combination of the compounds with low concentrations of dThd (25 μ M) resulted in a 2- to 7-fold decrease of the antiretroviral activity of the FddUrd analogues. Addition of uridine (Urd) (1000 μ M) did not remarkably affect the selectivity of FddUrd, FddBrUrd and FddIUrd. However, in the presence of Urd, the selectivity of FddClUrd decreased while that of AzddThd increased.

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Table 2. Anti-HIV effect of 5-halogen-substituted FddUrd derivatives

Compound	Upon addition of	ED ₅₀ ^a (μM)	CD ₅₀ ^b (μM)	S.I. ^c
FddUrd	-	0.04	0.95	24
	dThd (25 μM)	0.09	5.9	66
	dCyd (1000 μM)	7.6	79	10
	dThd (250 μM)+dCyd (1000 μM)	>500	>500	-
	Urd (1000 μM)	19	440	23
FddClUrd	-	0.38	535	1408
	dThd (25 μM)	2.5	442	177
	dCyd (1000 μM)	11	789	72
	dThd (250 μM)+dCyd (1000 μM)	52	500	10
	Urd (1000 μM)	5.2	>1000	>192
FddBrUrd	-	0.41	24	59
	dThd (25 μM)	0.76	79	104
	dCyd (1000 μM)	23	370	16
	dThd (250 μM)+dCyd (1000 μM)	140	135	0.96
	Urd (1000 μM)	4.7	468	100
FddIUrd	-	0.16	2.2	14
	dThd (25 μM)	0.26	1.7	7
	dCyd (1000 μM)	8.0	71	9
	dThd (250 μM)+dCyd (1000 μM)	360	>500	1.4
	Urd (1000 μM)	10	135	14
AzddThd	-	0.003	4.8	1600
	dThd (25 μM)	0.006	7.0	1167
	dCyd (1000 μM)	0.035	66	1886
	dThd (250 μM)+dCyd (500 μM)	29	>400	>14
	Urd (1000 μM)	0.02	134	6700

^a50% effective dose.^b50% cytotoxic dose.^cselectivity index or ratio of CD₅₀/ED₅₀.

Example 3

Inhibitory effects of FddClUrd, FddBrUrd, and FddIUrd on the expression of viral antigens in HIV-1-infected HUT-78 cells.

5 In a third set of experiments, anti-HIV-1 activity of the test compounds was also determined by monitoring viral antigen expression in HUT-78 cells at day 12 after HIV-1 infection. Indirect immunofluorescence, using a polyclonal antibody as probe, and laser flow cytofluorograph analysis (FACSTAR R, Becton Dickinson) were used for the
10 determination of antigen-positive cells. HUT-78 cells were infected with HIV-1 at 1000 CCID₅₀/ml and three quarters of the culture medium were replenished every 4th day.

As shown in Table 1 FddUrd was slightly more
15 effective as an antiviral agent than FddClUrd and FddBrUrd (ED₅₀ : 0.8, 1.6 and 2.4 μ M, respectively). FddIUrd was a less potent inhibitor of HIV-1 antigen expression in HUT-78 cells than FddUrd (ED₅₀ : 7.9 versus 0.8 μ M). AzddThd proved to be ~50-fold more effective than FddClUrd as an anti-HIV-1
20 agent in HUT-78 cells. It is noteworthy that none of the 5-halogeno-substituted FddUrd derivatives proved significantly cytostatic to HUT-78 cells at 50 μ M.

Example 4

25 Inhibitory effects of FddClUrd, FddBrUrd, and FddIUrd on HIV-1 induced cell fusion.

100 μ l of the test compounds were diluted in 200 μ l microplate wells of a Flat Bottom microtest III Plate (Falcon). Then 5×10^4 HIV-1-infected HUT-78 cells were
30 added to the wells, immediately followed by the addition of 5×10^4 Molt/8 cells, to yield a final volume of 200 μ l. The mixed cell culture was then incubated at 37°C in a CO₂-controlled humidified atmosphere. In this system, uninfected cells are able to interact with viral proteins,
35 expressed on the surface of the HIV-1-infected HUT-78 cells, leading to syncytia formation within a few hours of cocultivation. The first visible syncytia appear as soon as 2-5 hrs after cocultivation, and 20 hrs later an

abundant number of syncytia were present in the cell cultures.

None of the 5-halogeno-substituted FddUrd derivatives, including the parent FddUrd, and also FddThd and AzddThd, showed any protective effect to the coculture at a concentration as high as 50 μ M (data not shown).

Example 5

Transformation of C3H mouse embryo fibroblasts by Moloney murine sarcoma virus (MSV).

C3H cells were seeded at 20,000 cells per ml into wells of Costar Tissue Culture Cluster plates (48 wells per plate). Twenty-four hours later, cell cultures were infected by 80 foci-forming units of MSV during 120 min whereafter the culture medium was replaced by 1 ml fresh medium containing different concentrations of the test compounds. After 6 days, the transformation of the cell cultures was examined microscopically.

None of the 5-halogeno-substituted FddUrd derivatives, including the parent FddUrd, were endowed with a marked anti-MSV activity ($ED_{50} : >> 100 \mu$ M) (Table 1). In contrast, AzddThd and FddThd were extremely effective in inhibiting the retrovirus-induced transformation of the murine cells ($ED_{50} : 0.02$ and 0.06μ M, respectively).

Example 6

Inhibition of L1210/0, L1210/TK⁻, Raji/0 and Raji/TK⁻ cell proliferation.

All assays were performed in flat bottom Microtest III Plates (96 wells) (Falcon) as previously described. Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210 cells/well (200 μ l) or 7.5×10^4 Raji cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 and 96 hrs for L1210 cells, and 72 and 120 hrs for Raji cells at 37° in a humidified, CO₂-controlled atmosphere. At the end of the

incubation period, the cells were counted in a Coulter Counter (Coulter Electronics Ltd., Harpenden, Herts, U.K.). The 50% inhibitory dose (ID₅₀) was defined as the concentration of compound that reduced the number of cells by 50 %.

The proliferation of murine L1210/0 and L1210/TK⁻, and human Raji/0 and Raji/TK⁻ cells was not markedly affected by the test compounds at 500 μ M (Table 3), suggesting a cell-type dependent cytotoxic potential of the 5-halogeno-substituted Fdd analogues i.e., FddBrUrd and FddIUrd.

Table 3. Cytostatic effect of 5-halogeno-substituted FddUrd-derivatives

Compound	ID ₅₀ ^a (μ M)				
	L1210/0 ^b	L1210/TK ^{-b}	Raji/0 ^c	Raji/TK ^{-c}	MT-4 ^d
FddClUrd	> 500	> 500	> 500	> 500	> 500
FddBrUrd	> 500	> 500	> 500	> 500	> 500
FddIUrd	> 500	> 500	> 500	> 500	38
FddUrd	> 500	> 500	> 500	> 500	273
FddThd	-	-	-	-	27
AzddThd	-	-	-	-	49

^a50 % Inhibitory dose required to reduce the cell number by 50%.

^bSimilar values were obtained after 2 and 4 days of incubation.

^cSimilar values were obtained after 3 and 5 days of incubation.

^dValues obtained after 3 days of incubation.

Example 7

Interference of the 5-halogeno-substituted FddUrd derivatives with the phosphorylation of dThd by MT4 cell extracts.

- 5 FddClUrd, FddBrUrd, FddIUrd and FddUrd were evaluated on their potential to inhibit [methyl-³H]dThd phosphorylation by MT4 crude enzyme extracts (Table 4).
- dThd kinase was prepared from exponentially growing MT4 cells, which were first washed (2x) with
10 phosphate-buffered saline at 4°, suspended in buffer containing 10 mM potassium phosphate, pH 7.5 10 mM β -mercaptoethanol and 0.1 M KCl, and then homogenized at 25,000 g for 30 min. In the experiments, [methyl-³H]dThd served as the radiolabelled substrate. The apparent K_m and K_i
15 values were derived from Lineweaver-Burk plots, using a linear regression analysis program. The assay procedure has been described in detail (Balzarini, J. et al.; 5-Substituted 2'-deoxyuridines: correlation between inhibition of tumor cell growth and inhibition of thymidine kinase and thymidylate synthetase. Biochem. Pharmacol. 31:3673-3682 (1982)).

Table 4. Inhibition of MT4 dThd kinase by 5-halogeno-substituted FddUrd analogues

Compound	K_i (μM)	K_i/K_m^a	Type of inhibition
FddClUrd	3.14	5.74	competitive
FddBrUrd	3.86	5.21	competitive
FddIUrd	3.31	4.47	competitive
FddUrd	27.9	50.8	competitive

^a K_m values obtained in the individual experiments ranged from 0.6 to 1.1 μM .

Table 5. Inhibitory activity of 5-halogeno-substituted FddUrd analogues on tritium release from $[5-^3\text{H}]\text{dUrd}$ and $[5-^3\text{H}]\text{dCyd}$ in MT4 cells

Compound	ID_{50}^a (μM)	
	$[5-^3\text{H}]\text{dUrd}$	$[5-^3\text{H}]\text{dCyd}$
FddClUrd	287 \pm 100	>500
FddBrUrd	157 \pm 82	>500
FddIUrd	147 \pm 62	>500
FddUrd	\geq 500	>500
FddThd	29 \pm 17	>500
AzddThd	2.7 \pm 1.3	>500

^a50% inhibitory dose required to reduce tritium release by 50%.

The procedure to measure tritium release from (5-³H)dUrd or (5-³H)dCyd in intact cells has been described previously (Balzarini, J. et al. Strategies for the measurement of the inhibitory effect of thymidine analogs on the activity of thymidylate synthase in intact murine leukemia L1210 cells. Biochim. Biophys. Acta 785:36-45 (1984). Briefly, 10⁷ MT4 cells/ml were preincubated with an appropriate amount of test compound for 15 min at 37°. After this incubation period, radiolabelled (5-³H)dUrd or (5-³H)dCyd (100 uCi/ml; 0.1 uM) were added, and at various times (0, 15, 30, 45, 60 min), 100 ul of the reaction mixture was withdrawn, and mixed with 500 ul of cold suspension of carbon black (100 mg/ml) in 5 % TCA. After centrifugation at 1000 g for 10 min, supernatants were analysed for radioactivity.

All four compounds competitively inhibited the dThd kinase reaction. The K_i/K_m ratios were very similar for FddClUrd, FddBrUrd and FddIUrd (5.74, 5.21 and 4.47, respectively). Such low K_i/K_m values suggest a potent inhibitory effect of these compounds against dThd phosphorylation. Most likely, the 5-halogeno-substituted FddUrd, derivatives are good substrates for the cytosol dThd kinase. In this respect, the K_i/K_m values of these compounds are close to those for FddThd and only 4-5 times higher than those observed for AzddThd.

The differential affinities of the 5-halogeno-substituted FddUrd derivatives as well as FddUrd, FddThd and AzddThd for dThd kinase are closely correlated with their inhibitory effect on the intracellular tritium release from [5-³H]dUrd (Table 5). AzddThd and FddThd that were very good substrates for dThd kinase (Balzarini et al. The antiretroviral and cytostatic activity, and metabolism of 3'-azido-2',3'-dideoxythymidine, 3'-fluoro-2',3'-dideoxythymidine and 2',3'-dideoxycytidine are highly cell-type-dependent) were also the most potent

inhibitors of the tritium release from [5-³H]dUrd (ED₅₀ : 2.7 and 29 µM, respectively). FddClUrd, FddBrUrd and FddIUrd which inhibited (methyl-³H)dThd phosphorylation less efficiently than AzddThd and FddThd, proved also less inhibitory to tritium release from [5-³H]dUrd (ED₅₀ : 147-287 µM). FddUrd, the least potent inhibitor of dThd kinase did not affect tritium release from [5-³H]dUrd at

500 μ M. None of the test compounds evaluated affected tritium release from [5- 3 H]dCyd even at a concentration as high as 500 μ M (Table 5).

The examples indicate that the 5-halogeno-substituted derivatives of FddUrd are potent inhibitors of HIV-1 and HIV-2 replication in vitro. The ED₅₀ values for HIV-1 replication in MT4 cells ranked between 0.1 and 0.4 μ M. In this respect, they are 50- to 100-fold less effective than AzddThd when evaluated in the same in vitro system. However, there are striking differences in the toxicity of the compounds against MT4 cells. After 5 days of incubation, FddClUrd proved remarkably less cytotoxic to MT4 cells than the other FddUrd congeners, including FddThd and AzddThd. Consequently, the selectivity index (ratio 50 % cytotoxic dose/50 % effective dose) of FddClUrd markedly exceeded those of FddBrUrd, FddLUrd, FddUrd and FddThd by 1 to 2 orders of magnitude, and FddClUrd proved almost equally selective an anti-HIV-1 agent in MT4 cells than AzddThd.

With respect to their effect on syncytia formation none of the 5-halogeno-substituted FddUrd derivatives could prevent HIV-1-mediated cell fusion. In this respect the test compounds behaved like AzddThd, whose mechanism of anti-retroviral action is assumed to be due to a selective inhibition of reverse transcriptase (Furman, P.A., et al. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. Proc. Natl. Acad. Sci., USA, 83:8333-8337 (1986). Thus, the mechanism of antiretroviral action for the 5-halogeno-substituted FddUrd derivatives is most likely similar to that of AzddThd.

It is interesting to note that the 5-halogeno-substituted FddUrd derivatives including FddUrd are virtually inactive as inhibitors of MSV transformation of C3H cells. In contrast, FddThd and AzddThd are exquisitely effective as antiretroviral agents in this murine cell system. It is known that the 5'-triphosphate metabolite of FddUrd has a much less affinity for murine Rauscher leukemia virus reverse transcriptase than HIV reverse transcriptase; if these observations can be

extended to the 5-halogeno-substituted FddUrd analogues,
our findings for the 5-halogeno-substituted FddUrd
analogues can be explained.

WHAT WE CLAIM IS:

1. 5-Chloro-3'-fluoro-2',3'-dideoxyuridine.
2. 5-Bromo-3'-fluoro-2',3'-dideoxyuridine.
3. 5-Iodo-3'-fluoro-2',3'-dideoxyuridine.
4. 5-Fluoro-3'-fluoro-2',3'-dideoxyuridine.

5 5. A therapeutic composition for use in the treatment of retroviral diseases including hepatitis B which comprises as an active ingredient a 5-halogeno-3'-fluoro-2',3'-dideoxyuridine.

10 6. A therapeutic composition as claimed in claim 5 in which the active ingredient is selected from the group consisting of 5-chloro-3'-fluoro-2',3'-dideoxyuridine, 5-bromo-3'-fluoro-2',3'-dideoxyuridine, 5-iodo-3'-fluoro-dideoxyuridine and 5-fluoro-3'-fluoro-2',3'-dideoxyuridine.

15 7. A therapeutic composition as claimed in claim 5 comprising said active ingredient in a concentration ranging from about 0.1 - 100 % by weight.

20 8. A therapeutic composition as claimed in claim 7, having the form which is selected from the group consisting of powders, suspensions, solutions, sprays, emulsions, unguents and creams.

25 9. A therapeutic composition for use in the treatment of AIDS or AIDS-related diseases which comprises as an active ingredient a 5-halogeno-3'-fluoro-2',3'-dideoxyuridine.

30 10. A therapeutic composition as claimed in claim 9 in which the active ingredient is selected from the group consisting of 5-chloro-3'-fluoro-2',3'-dideoxyuridine, 5-bromo-3'-fluoro-2',3'-dideoxyuridine, 5-iodo-3'-fluoro-2',3'-dideoxyuridine and 5-fluoro-3'-fluoro-2',3'-dideoxyuridine.

35 11. A therapeutic composition as claimed in claim 9 comprising said active ingredient in a concentration ranging from about 0.1 - 100 % by weight.

12. A therapeutic composition as claimed in claim 11, having the form which is selected from the group consisting of powders, suspensions, solutions, sprays, emulsions, unguents and creams.

13. A method for the treatment of a retroviral disease including hepatitis B which comprises administering to a patient suffering from the retroviral disease a 5-halogeno-3'-fluoro-2',3'-dideoxyuridine.

5 14. A method as claimed in claim 13 in which the 5-halogeno-3'-fluoro-2',3'-dideoxyuridine is selected from the group consisting of 5-chloro-3'-fluoro-2',3'-dideoxyuridine, 5-bromo-3'-fluoro-2',3'-dideoxyuridine, 5-iodo-3'-fluoro-2',3'-dideoxyuridine and 5-fluoro-3'-fluoro-2',3'-dideoxyuridine.

10 15. A method for the treatment of AIDS or AIDS-related diseases, which comprises administering to a patient suffering from AIDS and AIDS-related diseases a 5-halogeno-3'-fluoro-2',3'-dideoxyuridine.

15 16. A method as claimed in claim 15 in which the 5-halogeno-3'-fluoro-2',3'-dideoxyuridine is selected from the group consisting of 5-chloro-3'-fluoro-2',3'-dideoxyuridine, 5-bromo-3'-fluoro-2',3'-dideoxyuridine, 5-iodo-3'-fluoro-2',3'-dideoxyuridine and 5-fluoro-3'-fluoro-2',3'-dideoxyuridine.

20 17. The use of 5-halogeno-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against a retroviral disease including hepatitis B.

25 18. The use of 5-chloro-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against a retroviral disease including hepatitis B.

19. The use of 5-bromo-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against a retroviral disease including hepatitis B.

30 20. The use of 5-iodo-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against a retroviral disease including hepatitis B.

21. The use of 5-fluoro-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against a retroviral disease including hepatitis B.

35 22. The use of 5-halogeno-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against AIDS or AIDS-related diseases.

23. The use of 5-chloro-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against AIDS

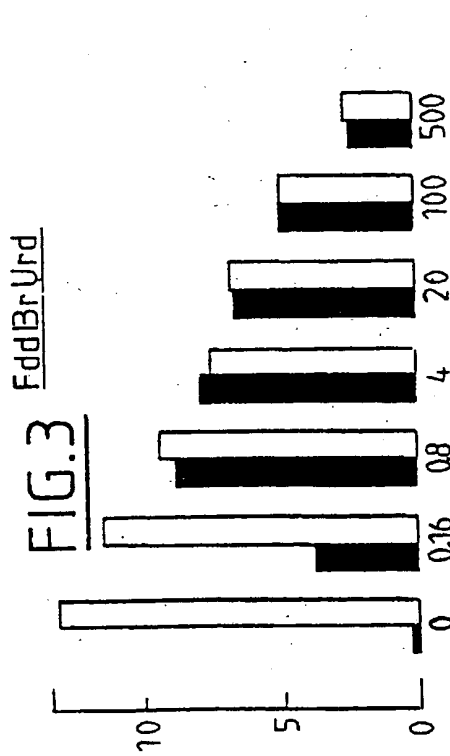
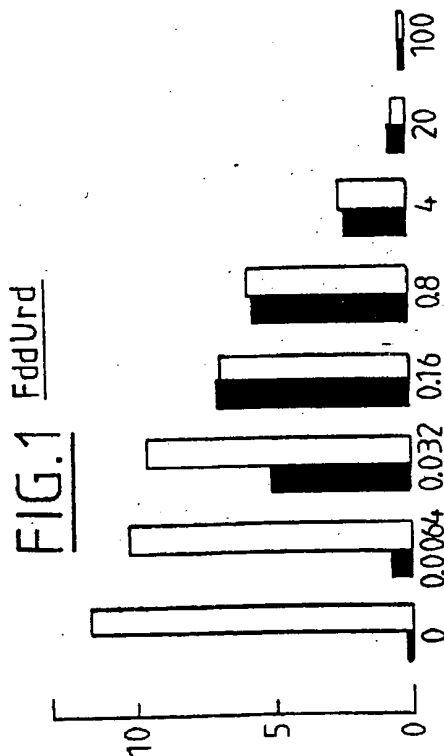
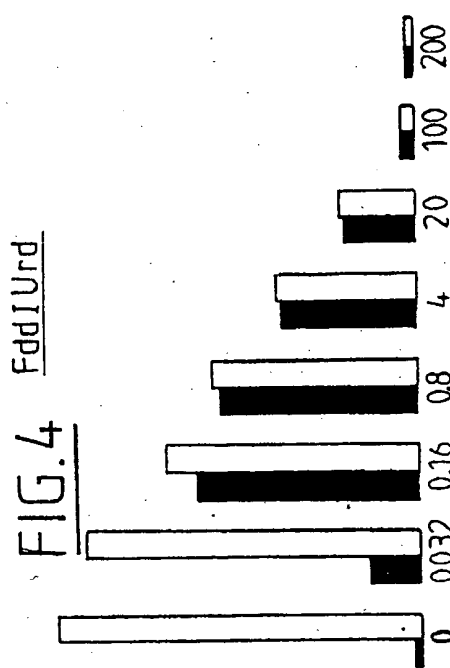
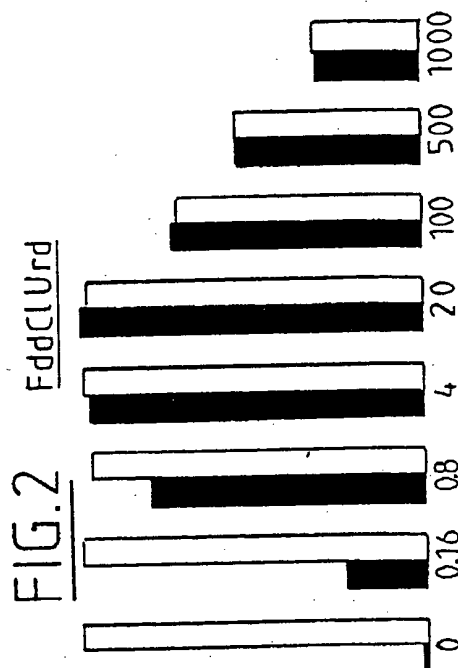
or AIDS-related diseases.

24. The use of 5-bromo-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against AIDS or AIDS-related diseases.

5 25. The use of 5-iodo-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against AIDS or AIDS-related diseases.

10 26. The use of 5-fluoro-3'-fluoro-2',3'-dideoxyuridine for preparing a therapeutic composition against AIDS or AIDS-related diseases.

1/1



Concentration of compound (μM)

Viable cell number (x10⁵/ml)

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 89/01180

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 H 19/06, 19/073; A 61 K 31/70														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched †</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">IPC5</td> <td style="padding: 5px;">C 07 H; A 61 K</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *</div>			Classification System	Classification Symbols	IPC5	C 07 H; A 61 K								
Classification System	Classification Symbols													
IPC5	C 07 H; A 61 K													
III. DOCUMENTS CONSIDERED TO BE RELEVANT* <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category *</th> <th style="width: 60%; padding: 5px;">Citation of Document, †† with Indication, where appropriate, of the relevant passages ‡</th> <th style="width: 30%; padding: 5px;">Relevant to Claim No. ‡</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">EP, A2, 0254268 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 27 January 1988, see the whole document --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-12, 17-26</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">Chemical Abstracts, volume 100, no. 3, 16 January 1984, (Columbus, Ohio, US), Ajmera, Sudhir et al. : "Synthesis and biological activity of 5-fluoro-2',3'- dideoxy-3'-fluorouridine and its 5'-phosphate ", see page 242, abstract 19719w, & J. Med. Chem. 1984, 27(1), 11- 14 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-12, 17-26</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">US, A, 3775397 (GERHARD ETZOLD ET AL.) 27 November 1973, see particularly claim 1 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-12</td> </tr> </tbody> </table>			Category *	Citation of Document, †† with Indication, where appropriate, of the relevant passages ‡	Relevant to Claim No. ‡	X	EP, A2, 0254268 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 27 January 1988, see the whole document --	1-12, 17-26	X	Chemical Abstracts, volume 100, no. 3, 16 January 1984, (Columbus, Ohio, US), Ajmera, Sudhir et al. : "Synthesis and biological activity of 5-fluoro-2',3'- dideoxy-3'-fluorouridine and its 5'-phosphate ", see page 242, abstract 19719w, & J. Med. Chem. 1984, 27(1), 11- 14 --	1-12, 17-26	X	US, A, 3775397 (GERHARD ETZOLD ET AL.) 27 November 1973, see particularly claim 1 --	1-12
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: †</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 50%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search 21st December 1989</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em; font-weight: bold;">08 JAN. 1990</div></td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div></td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer <div style="text-align: right; font-weight: bold; margin-top: 10px;">T.K. WILLIS</div></td> </tr> </table>			Date of the Actual Completion of the International Search 21st December 1989	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em; font-weight: bold;">08 JAN. 1990</div>	International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: right; font-weight: bold; margin-top: 10px;">T.K. WILLIS</div>								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	EP, A2, 0317128 (THE WELLCOME FOUNDATION LIMITED) 24 May 1989, see the whole document	1-12,17- 26
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P,X	EP, A2, 0305117 (THE WELLCOME FOUNDATION LIMITED) 1 March 1989, see the whole document	1-12,17- 26
	--	
P	Chemical Abstracts, volume 111, no. 7, 14 August 1989, (Columbus, Ohio, US), Balzarini Jan et al.: "5-Halogeno-3'-fluoro-2',3'-dideoxyuridines as in hibitors of human immunodeficiency virus (HIV): potent and selective anti-HIVactivity of 3'-fluoro-2',3'-dideoxy-5-chloro uridine.", see page 22, abstract 49972t, & Mol. Pharmacol. 1989, 35(5), 571-7	1-12,17- 26
	--	
P	Chemical Abstracts, volume 111, no. 7, 14 August 1989, (Columbus, Ohio, US), Van Aerschot A.: "3'-Fluoro-2',3'-dideoxy-5-chlorouridine: most selective anti-HIV-1 agent among a series of new 2'- and 3'-fluorinated 2',3'-dideoxynucleoside analogs ", see page 810, abstract 58257r, & J. Med. Chem. 1989, 32(8), 1743-1749	1-12,17- 26
	--	
Y	GB, A, 2181128 (THE WELLCOME FOUNDATION LIMITED) 15 April 1987, see the whole document, particularly examples 31-33	1-12,17- 26
	--	
Y	J. Med. Chem., Vol. 30, 1987, Piet Herdewijn et al.: "3'-substituted 2',3'-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents ", see page 1270-1278 see the whole article, particularly Scheme I	1-12,17- 26
	--	
X	Chemical Abstracts, volume 78, no. 21, 28 May 1973, (Columbus, Ohio, US), Cech D. et al.: "Reaction of uracil and its nucleosides with elemental fluorine. ", see page 414, abstract 136580g, & J. Prakt. Chem. 1973, 315(1), 149-154	1-12

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 13-16 because they relate to subject matter not required to be searched by this Authority, namely:

Method for treatment of the human or animal body by therapy
(Rule 39 PCT).

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

PCT/EP 89/01180

SA

31510

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EPP file on 08/11/89. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family - member(s)	Publication date
EP-A2- 0254268	27/01/88	JP-A- 63107936 AU-D- 12592/88	12/05/88 10/11/88
US-A- 3775397	27/11/73	NONE	
EP-A2- 0317128	24/05/89	AU-D- 24763/88 JP-A- 1153697	25/05/89 15/06/89
EP-A2- 0305117	01/03/89	AU-D- 21429/88 JP-A- 1068325	25/05/89 14/03/89
GB-A- 2181128	15/04/87	AU-D- 62702/86 EP-A- 0217580 EP-A-B- 0196185 DE-A- 3608606 JP-A- 62103100 AU-A- 572019 CA-A- 1238277 EP-A- 0291633 JP-A- 63290895 DE-A- 3645059 EP-A- 0306597 DE-A- 3645058 US-A- 4818750 US-A- 4847244 DE-A- 3664257 US-A- 4857511	19/03/87 08/04/87 01/10/86 18/09/86 13/05/87 28/04/88 21/06/88 23/11/88 28/11/88 05/01/89 15/03/89 02/02/89 04/04/89 11/07/89 17/08/89 15/08/89

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